CLAIM OR CLAIMS

WE CLAIM:

1) A method for detecting a target nucleic acid sequence, the method comprising:

a) providing one or more target probes comprising a linear single-stranded DNA molecule, the target probes comprising at least two target-complementary sequences that are not joined to each other, wherein the 5'-end of a first target-complementary sequence is complementary to the 5'-end of the target nucleic acid sequence, and wherein the 3'-end of a second target-complementary sequence is complementary to the 3'-end of the target nucleic acid sequence, and wherein the target probe that comprises the first target-complementary sequence also comprises a promoter that is joined to the 3'-of the first target-complementary sequence, wherein the promoter binds an RNA polymerase that lacks helicase-like activity and that can transcribe RNA using a single-stranded promoter;

b) contacting the target probes with the target nucleic acid sequence and incubating under hybridization conditions, such that the target-complementary sequences anneal adjacently to the target nucleic acid sequence to form a target probe-target complex;

c) contacting the target probe-target complex with a ligase under ligation conditions to form a transcription substrate;

d) contacting the transcription substrate with the RNA polymerase;

e) optionally, repeating steps (a) through (e); and

f) detecting the transcription product.

2) The method of claim 1, wherein the target nucleic acid sequence comprises a single-stranded DNA molecule obtained by reverse transcription of RNA.

3) The method of claim 1, wherein the target nucleic acid sequence comprises a DNA target nucleic acid in a sample.

4) The method of claim 1, wherein the method is used for detecting an analyte in a sample, wherein the target nucleic acid sequence comprises a target sequence tag that is joined to an analyte-binding substance, the method further comprising prior to step (a) contacting the analyte-binding substance with the analyte to form a specific binding pair and separating the specific binding pair from analyte-binding substance molecules that are not bound to the analyte.

5) The method of claim 4, wherein the analyte is selected from the group consisting of a biochemical molecule, a biopolymer, a protein, a glycoprotein, a lipoprotein, an enzyme, a hormone, a biochemical metabolite, a receptor, an antigen, an antibody, a nucleic acid, a DNA molecule, an RNA molecule, a polysaccharide and a lipid.

6) The method of claim 4, wherein the analyte-binding substance is selected from the group consisting of a nucleic acid, a polynucleotide, an oligonucleotide, a segment of a nucleic acid or polynucleotide, a DNA molecule, an RNA molecule, a molecule comprising both DNA and RNA mononucleotides, modified DNA mononucleotides, a molecule obtained by a method termed "SELEX", a nucleic acid molecule having an affinity for protein molecules, a polynucleotide molecule having an affinity for protein molecules, an operator, a promoter, an origin of replication, a ribosomal nucleic acid sequence, a sequence recognized by steroid hormone-receptor complexes, a peptide nucleic acid (PNA), a nucleic acid and a PNA, a molecule prepared by using a combinatorial library of randomized peptide nucleic acids, an oligonucleotide or polynucleotide with a modified backbone that is not an amino acid, a lectin, a receptor for a hormone, a hormone, and an enzyme inhibitor.

7) The method of claim 1, wherein the target nucleic acid sequence comprises a DNA target nucleic acid that is a product of an amplification reaction.

8) The method of claim 1 wherein the target nucleic acid sequence comprises a product of rolling circle replication.

9) The method of claim 7, wherein the amplification reaction is selected from the group consisting of PCR, RT-PCR, NASBA, TMA, 3SR, LCR, LLA, SDA, Multiple Displacement Amplification, ICAN™, UCAN™, Loop-AMP, SPIA™ and Ribo-SPIA™.

10) The method of claim 1, wherein the one or more target probes comprise a bipartite target probe.

11) The method of claim 1 wherein the target probe comprising the second target-complementary sequence also comprises a signal sequence 5'-of said target-complementary sequence.

12) The method of claim 11 wherein the signal sequence comprises a substrate for Q-beta replicase.

13) The method of claim 11 wherein the signal sequence comprises a sequence that encodes a detectable protein.

14) The method of claim 13 wherein the detectable protein is green fluorescent protein.

15) The method of claim 11 wherein the signal sequence comprises a sequence that is detectable by a probe.

16) The method of claim 11 wherein the signal sequence comprises a sequence that is detectable by a molecular beacon.

17) The method of claim 10 wherein the bipartite target probe comprises a transcription termination sequence 5'-of the second target-complementary sequence.

18) The method of claim 10 wherein the bipartite target probe comprises two target-complementary sequences that can anneal adjacently to the target nucleic acid sequence.

19) The method of claim 1, wherein the one or more target probes comprise a promoter target probe comprising the first target-complementary sequence and a signal target probe comprising the second target-complementary sequence.

20) The method of claim 19 wherein the signal target probe comprises a signal sequence 5'-of the second target-complementary sequence.

21) The method of claim 20 wherein the signal sequence comprises a substrate for Q-beta replicase.

22) The method of claim 20 wherein the signal sequence comprises a sequence that encodes a detectable protein.

23) The method of claim 22 wherein the detectable protein is green fluorescent protein.

24) The method of claim 20 wherein the signal sequence comprises a sequence that is detectable by a probe.

25) The method of claim 20 wherein the signal sequence comprises a sequence that is detectable by a molecular beacon.

26) The method of claim 19 wherein the first target-complementary sequence and the second target-complementary sequence can anneal adjacently to the target nucleic acid sequence.

27) The method of claim 1 wherein one target probe is provided.

28) The method of claim 1 wherein two target probes are provided.

29) The method of claim 1 wherein the number of provided target probes is selected from the group consisting of 3, 4, 5, 6, 7, 8, 9, and 10.

30) The method of claim 1 wherein the promoter is an N4 vRNAP promoter set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

31) The method of claim 1 wherein the promoter is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

32) The method of claim 1 wherein the ligase is Ampligase® Thermostable DNA Ligase.

33) The method of claim 1 wherein the ligase is selected from the group consisting of Tfl DNA Ligase, Tsc DNA Ligase, *Pfu* DNA ligase, T4 DNA ligase and Tth DNA ligase.

34) The method of claim 1 wherein the RNA polymerase comprises a region encoding a polypeptide having an amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:15.

35) The method of claim 1 wherein the RNA polymerase comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:14.

36) The method of claim 1 wherein the RNA polymerase comprises an N4 min-vRNAP.

37) The method of claim 1 wherein the RNA polymerase comprises a domain having 1,106 amino acid residues corresponding to amino acid residues 998-2103 of N4 vRNAP.

38) The method of claim 1 wherein the transcription product comprises only ribonucleotides.

39) The method of claim 1 wherein the transcription product comprises at least one pyrimidine 2'-deoxyribonucleotide having a 2'-substituent on the sugar moiety.

40) The method of claim 1 wherein the transcription product comprises at least one pyrimidine 2'-fluoro-2'-deoxyribonucleotide.

41) The method of claim 1 wherein the transcription product comprises pyrimidine 2'-fluoro-2'-deoxyribonucleotides.

42) The method of claim 1 wherein the transcription product comprises AMP, GMP, 2'-F-dUMP and 2'-F-dCMP.

43) The method of claim 1 wherein the transcription product comprises at least one 2'-amino-2'-deoxyribonucleotide.

44) The method of claim 1 wherein the transcription product comprises at least one 2'-methoxy-2'-deoxyribonucleotide.

45) The method of claim 1 wherein the transcription product comprises at least one 2'-azido-2'-deoxyribonucleotide.

46) The method of claim 1 wherein the transcription product comprises at least one 2'-amino-2'-deoxyribonucleotide.

47) A method for detecting a target nucleic acid sequence, the method comprising:

a) providing a target sequence amplification probe (TSA probe) comprising a linear single-stranded DNA molecule comprising a 5'-end portion and a 3'-end portion that are not joined, wherein the 5'-end portion is complementary to the 5'-end of the target nucleic acid sequence, and wherein the 3'-end portion is complementary to the 3'-end of the target nucleic acid sequence;

b) providing a primer that is complementary to the TSA probe;

c) providing one or more target probes comprising a linear single-stranded DNA molecule, the target probes comprising at least two target-complementary sequences that are not joined to each other, wherein the 5'-end of a first target-complementary sequence is complementary to the 5'-end of the target nucleic acid sequence, and wherein the 3'-end of a second target-complementary sequence is complementary to the 3'-end of the target nucleic acid sequence; and wherein the target probe that comprises the first target-complementary sequence also comprises a promoter that is joined to the 3'-of the first target-complementary sequence, wherein the promoter binds an RNA polymerase that lacks helicase-like activity and that can transcribe RNA using a single-stranded promoter;

d) contacting the TSA probe with the target nucleic acid sequence and incubating under hybridization conditions, such that the end portions anneal adjacently to the target nucleic acid sequence to form a TSA probe-target complex;

e) contacting the TSA probe-target complex with a ligase under ligation conditions, such that a target sequence amplification circle (TSA circle) is formed;

f) contacting the TSA circle with the primer and incubating under hybridization conditions to form a TSA circle-primer complex;

g) contacting the TSA circle-primer complex with a strand-displacing DNA polymerase under strand-displacing polymerization conditions, such that a rolling circle replication product comprising multiple copies of the target nucleic acid sequence is formed;

h) contacting the target probes with the rolling circle replication product and incubating under hybridization conditions, such that the target-complementary sequences anneal adjacently to the rolling circle replication product to form a rolling circle replication product-target complex;

i) contacting the rolling circle replication product-target complex with a ligase under ligation conditions to form a transcription substrate;

j) optionally, releasing the transcription substrate from the rolling circle replication product complex,

k) contacting the transcription substrate with the RNA polymerase under transcription conditions to form a transcription product;

l) optionally, repeating steps (a) through (k); and

m) detecting the transcription product.

48) A method for selectively transcribing a target nucleic acid sequence, the method comprising a DNA ligation operation and a transcription operation, wherein the DNA ligation operation comprises ligation of one or more target probes comprising a promoter that is 3'-of a target complementary sequence, which promoter binds an RNA polymerase that lacks helicase-like activity and that can transcribe RNA using a single-stranded promoter to form a transcription substrate, wherein the ligation is dependent on hybridization of the target probes to the target nucleic acid sequence, to form a transcription substrate and wherein the transcription operation comprises contacting the transcription substrate with the RNA polymerase.

49) A method for detecting a target nucleic acid sequence, the method comprising:

a) providing one or more target probes comprising a linear single-stranded DNA molecule, the target probes comprising at least two target-complementary sequences that are not joined to each other, wherein the 5'-end of a first target-complementary sequence is complementary to the 5'-end of the target nucleic acid sequence, and wherein the 3'-end of a second target-complementary sequence is complementary to the 3'-end of the target nucleic acid sequence, and wherein the target probe that comprises the first target-complementary sequence also comprises a promoter that is joined to the 3'-end of the first target-complementary sequence, which promoter binds an RNA polymerase that lacks helicase-like activity and that can transcribe RNA using a single-stranded promoter;

b) contacting the target probes with the target nucleic acid sequence and incubating under hybridization conditions, such that the target probes anneal to the target nucleic acid sequence to form a target probe-target complex;

c) contacting the target probe-target complex with a DNA polymerase under DNA polymerization conditions to form one or more DNA polymerase extension products that are adjacent to the 5'-end of a target-probe, such that a complex is formed;

d) contacting the complex with a ligase under ligation conditions to form a transcription substrate;

e) contacting the transcription substrate with the RNA polymerase to form a transcription product;

f) optionally, repeating steps (a) through (f); and

g) detecting the transcription product.

50) A kit for detecting a target nucleic acid sequence, the kit comprising:

a) one or more target probes comprising a linear single-stranded DNA molecule, the target probes comprising at least two target-complementary sequences that are not joined to each other, wherein the 5'-end of a first target-complementary sequence is complementary to the 5'-end of the target nucleic acid sequence, and wherein the 3'-end of a second target-complementary sequence is complementary to the 3'-end of the target nucleic acid sequence, and wherein the target probe that comprises the first target-complementary sequence also comprises a promoter that is joined to the 3'-of the first target-complementary sequence, wherein the promoter binds an RNA polymerase that lacks helicase-like activity and that can transcribe RNA using a single-stranded promoter;

b) a ligase; and

c) the RNA polymerase.

51) The kit of claim 50 further comprising a reverse transcriptase.

52) The kit of claim 50 further comprising a target sequence amplification probe (TSA probe) comprising a linear single-stranded DNA molecule comprising a 5'-end portion and a 3'-end portion that are not joined, wherein the 5'-end portion is complementary to the 5'-end of the target nucleic acid sequence, and wherein the 3'-end portion is complementary to the 3'-end of the target nucleic acid sequence; a primer that is complementary to the target sequence amplification probe, and a strand-displacing DNA polymerase.

53) The kit of claim 50 further comprising a DNA polymerase.